A Polypropylene sulfide nanoparticle p24 vaccine promotes dendritic cell-mediated specific immune responses against HIV-1

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Abstract

Delivery of vaccine formulations into the dermis using antigen-coated microneedle patches is a promising and safe approach due to efficient antigen delivery and safety. We have evaluated an intradermal vaccine using HIV-1 p24 Gag peptide-conjugated polypropylene sulfide nanoparticles (PPS-NP) to induce immunity against HIV-1. This PPS-NP formulation did not accelerate the maturation of blood- or skin-derived subsets of dendritic cells (DCs), either generated *in vitro* or purified *ex vivo*, despite efficient uptake in the absence of adjuvant. Moreover, DC-mediated capture of particulate antigen in this form induced potent HIV-1-specific $CD4^+$ T cell responses, as well as B cell-mediated antibody production. Nanoparticle-based intradermal antigen delivery may therefore provide a new option in the global effort to develop an effective vaccine against HIV-1.

Introduction

Despite major advances in antiretroviral therapy and pre- and post-exposure prophylaxis measures, the HIV-1 pandemic remains a major threat to global health. The promising results of the Thai trial RV144 have raised interest in the development of a transmission-preventive vaccine that elicits potent neutralizing antibodies against a broad spectrum of HIV-1 strains (Doria-Rose et al., 2014; Haynes et al., 2012).

Direct targeting of antigens to DC and Langerhans cells (LC) in the skin or lymph nodes is a strategy that has considerable potential to induce effective immune responses (Liard et al., 2012; Seneschal et al., 2012). DC are major antigen presenting cells (APCs) forming a complex network bridging non-lymphoid tissues to T cell areas of secondary lymphoid organs. LC home to epithelial surfaces, where they remain in a resting immature state until antigen encounter (Geissmann et al., 2002). Immature resident DC/LC have a very active endocytic system that is highly coordinated for efficient antigen processing (Turley et al., 2000). Upon exposure to external stimuli, cytokines, bacterial DNA or double stranded RNA, LC/DC become activated and reach a mature antigen presenting state. Within local draining lymphoid organs, antigen-activated LC/DC interact with antigen-specific T cells and initiate immune responses. Using *in vitro* models recapitulating the early events of HIV infection, DC and LC were shown to play a crucial role in HIV-1 transfer to CD4+ T lymphocytes, as well as in the generation of immune responses against HIV-1 (Piguet and Blauvelt, 2002; Piguet et al., 2014; Piguet and Steinman, 2007).

It is well established that intradermal (ID) injection delivers antigens to the skin DC and subsequently the proximal draining lymph node (Liard et al., 2012; Rancan et al., 2014). In recent years microneedle devices have also been used for the minimally invasive delivery of antigens to the epidermal and dermal skin compartments (Pearton et al., 2010; Song et al., 2010). Microneedles offer the advantage of reduced needle size which translates to less pain, less risk of transmission of blood borne pathogens, easier disposal and more refined targeting to the epidermis and dermis (Pearton et al., 2013). Nanoparticle technology has also shown to improve targeted delivery of antigens to the lymph node (Reddy et al., 2007). With this method, DC take up particulate antigens efficiently and immune responses appear strong and sustained. Moreover, particulate

antigens can induce CD8+ cytotoxic T lymphocyte responses via peptide cross presentation onto major histocompatibility complex (MHC) class I molecules. Complexing antigens with nanoparticles offers many advantages such as preservation from proteolytic enzymes, favoring antigen distribution to APCs, improved shelf-life of the antigen for long term storage, and suitability for large-scale pharmaceutical production.

In this study, we have used biocompatible, degradable polymer nanoparticles (NP) to deliver antigen. Briefly, poly(propylene sulfide) (PPS) NPs were formed by emulsion polymerization of propylene sulfide using the block copolymer pluronic as an emulsifier (Hirosue et al., 2010; Reddy et al., 2006). The resulting NP consists of a poly(propylene sulfide) core, with a hydrophilic poly(ethylene glycol) corona, derived from the pluronic emulsifier. Using the disulfide link on the activated NP surface, we conjugated a p24 peptide from the HIV-1 structural protein Gag to make p24NP. The immunogenic properties of this p24NP formulation were then tested in relation to skin immunization, DC maturation, DC-mediated antigen-driven T cell stimulation and antibody production.

Results

p24NP is rapidly taken up by DC and does not induce early maturation

Monocyte-derived dendritic cells (MDDC) were incubated with two different doses of p24NP and collected at various time points up to 24h. Intracellular staining for p24 HIV capsid protein was then performed to evaluate the kinetics of NP-conjugated p24 uptake by DC at various time points. As shown in Fig. 1a, p24 staining was detected by flow cytometry as early as 30 min after exposure and increased with time, indicating rapid and efficient uptake of p24NP by DC. Interestingly, no significant differences in p24NP uptake were observed between the two different doses, suggesting that internalization efficiency was already maximal at a concentration of 0.1 μg/ml.

As our p24NP formulation was non-adjuvanted, we wanted to exclude the induction of early DC maturation. Using CD83 cell surface expression and TNF-α production as markers, we checked DC phenotype and cytokine production when DC pulsed with p24NP compared to unconjugated NP. MDDC were treated overnight with various concentrations of unconjugated NP as control or p24NP ± 1μg/ml lipopolysaccharide (LPS) adjuvant overnight and stained for CD83 surface expression, a typical marker of mature DC (Zhou et al., 1992). Neither unconjugated NP nor p24NP alone modulated CD83 expression, while LPS clearly induced DC maturation (Fig. 1b). In parallel, we measured the intracellular levels of TNF-α, one of the major pro-inflammatory cytokines produced by DC after LPS stimulation. Little to no TNF-α production was detected in response to NP or p24NP (Fig. 1b, c). In contrast, more than 50% of DC significantly produced TNF-α when NP or p24NP were used in combination with LPS, therefore confirming that our NP formulation would not contain any adjuvant properties.

To further explore our system, we analyzed in parallel MDDC with dermal-like DC – monocyte-derived DC differentiated in the presence of Vitamin D3 (Chu et al., 2012; Nestle et al., 1993). Similarly, p24NP uptake by MDDC or CD141+ dermal-like DC did not induce early maturation or activation (Fig. 1d). Of note, upregulation of CD83 cell surface expression and TNF-α production was observed when DC were treated with other adjuvants such as R848 and poly(I:C) (data not shown).

p24NP-loaded DC induce specific IFN-γ anti-p24 specific response in CD8 and CD4 T cell clones

To assess the capacity of blood-derived DC to induce specific immune responses following p24NP uptake, we utilized CD8 T cells clones specific for the HLA-B57-restricted of HIV-1 p24 Gag TW10 epitope (108-117) and assessed their secretion of IFN-γ (Feeney et al., 2005). MDDC and dermal-like DC from HLA-B57+ donors were pulsed with NP alone, p24 peptide or p24NP for 4h, then cocultured overnight with TW10-specific CD8+ T cells. IFN-γ production was measured by intracellular staining and analyzed in CD3+ cell by flow cytometry. As shown in representative experiments with MDDC (Fig. 2a) and dermal-like DC (Fig. 2b), no IFN-γ production was detected when DC were pulsed with NP alone, whereas CD8+ T cells produced large quantities of IFN-γ when treated with phorbol myristyl acetate (PMA). In contrast, DCs loaded with p24 peptide or p24NP induced IFN-γ production by TW10-specific $CD8^+$ T cells in a dose-related manner (Fig. 2c). Interestingly, TW10-specific CD8+ T cell responses were more potent in the presence of p24NP compared to unconjugated p24 peptide at most concentrations, especially with dermal-like DCs (Fig. 2a-c). Although individual values were statistically different, difference between both p24 and p24NP curves was not significant.

Similarly, we tested p24NP formulation efficiency to stimulate CD4+ T cells in DC cocultures with HLA-DR1-restricted HIV-1 p24 Gag (294-313)-specific CD4 T cell clone Ox97-clone 10 (Scriba et al., 2005). MDDC or dermal-like DC from selected HLA-DR1+ donors were treated with p24 or p24NP and cocultured overnight with Ox97 CD4 T cells. As for CD4+ T cell activation, the efficiency of p24NP, compared to p24 peptide alone, in boosting CD4+ T cell response could be observed when dermal-like DC (Fig. 3a and c) or MDDC (Fig. 3b and d) were pulsed with suboptimal concentrations of nanoparticles ranging from 0.01 to 0.5 μg/ml. However, these responses were enhanced in the nanoparticle formulation relative to p24 peptide alone at suboptimal concentrations (0.01 to 0.5 μg/ml), suggesting that this lowered sensitivity threshold may reflect a

greater degree of antigen preservation in DC processing p24NP. Nonetheless, differences showed in Fig. 3c and 3d between p24 and p24NP were not statistically significant.

p24NP priming induces CD4 T cell response to HIV-1 infection

Because our proposed formulation for vaccine delivery aims at inducing a broad and sustained adaptive immune response, we examined the level of polyclonal CD4+ T cells activation upon co-culture with MDDC or monocyte-derived Langerhans cells (MDLC), as models of mucosal and skin resident DC subsets. Accordingly, CD4+ T cells were stimulated with p24/p24NP-loaded autologous MDDC or MDLC for 2 rounds and restimulated. As shown in Fig. 4b, polyclonal CD4+ T cells IFN-γ response was higher when MDLC were pre-loaded with p24NP compared to p24 peptide (11.2% vs. 3.1%). A similar increase was observed with MDDC (16.8% of IFN-γ+/CD3+ in the p24NP condition compared to 6.8% with p24 peptide) (Fig. 4b, lower panels). Statistical analysis in Fig. 4a shows significant enhancement of CD4+ T cells IFN-γ production when p24NP is used compared to p24, independently of the type of DC used.

To further analyze the potential of our p24NP formulation as a vaccine and to generate an optimal adaptive immune response against HIV-1, autologous CD4+ T cells were stimulated for 6 days with p24- or p24NP-loaded whole blood mature DCs. Cells were then washed and co-cultured for another 6 days with HIV-1-infected MDLC or MDDC before analysis of CD4+ T cell IFN-γ and IL-2 production (Fig. 4c). Surprisingly, we observed that specific CD4+ T cells responses against HIV-1 were similar in co-culture with HIV-1-infected MDLC primed with p24NP or p24 (12.6% vs. 9.5% respectively). However, in co-culture with HIV-1 infected MDDC, CD4+ T cells responses were significantly greater when primed with p24NP-loaded MDDC compared to p24-loaded MDDC (7.6% vs. 2.4%) (Fig. 4c, lower panels). Similar patterns emerged when we examined the production of IL-17, a cytokine critically involved in vaccine-induced mucosal immunity (Khader et al., 2009). IL-17 production was similar upon re-stimulation with MDLC, while an increased IL-17 production was observed in p24NP-primed CD4+ T cell co-cultured with HIV-1-infected MDDC (28.7%) compared to CD4+ T cells primed with p24-loaded MDDC (8.8%) (Fig. 4d). Collectively, these data

suggest that CD4+ T cell polyclonal response to HIV-1 infection is enhanced when primed with p24NP-loaded DC compared to p24-loaded DC.

Antibody response to p24NP

An important goal of vaccine strategy relies on the efficient induction of humoral response (Steinman and Pope, 2002). In response to stimulation with antigen-exposed DC, B cells are primed to produce specific antibodies (Streeck et al., 2013; Walker et al., 2011). In order to assess the antibody response to p24NP immunization, primary CD4+ T lymphocytes were stimulated with p24NP-loaded autologous DC, and then cocultured with autologous B cells for 2 weeks. Supernatants were harvested and antibody production tested by ELISA. As shown in Fig. 5a, an average of 910 ng/ml IgG1 and 660 ng/ml IgG2 are produced in response to p24NP immunization compared to 1,460 ng/ml IgG1 and 795 ng/ml IgG2 in response to p24 suggesting that p24NP formulation can generate potent antibody response as a result of an optimal DC-mediated CD4+ stimulation (differences are not statistically significant).

p24NP is delivered into skin-resident DC and stimulates CD4+ T cells

To determine whether our NP-based vaccine formulation is effectively targeted to relevant immune cells via intradermal delivery, surrogate fluorescent NP-coated microneedles were inserted into skin explants and maintained in place for 60 sec. Using fluorescence microscopy, we observed that injected fluorescent nanobeads (yellow) were efficiently and exclusively delivered into the dermal layers, slightly deeper than the CD207+ LC (stained in red)-containing epithelial layer (Fig. 5b, c).

We next sought to evaluate the effect of p24NP on *ex vivo* isolated DC subsets from skin samples. Skin DC were isolated from dermis and epidermis by walk-out and treated with p24NP, p24, NP only or LPS. As anticipated, DC treated with NP, p24NP or p24 peptide did not produce significant levels of TNF-α, while LPS induces TNF-α production (Fig. 6a) and DC maturation (data not shown), therefore excluding a lack of functional responsiveness in these isolated skin DC.

To analyze the subsequent CD4+ T cell responses to *ex vivo* loaded DC, we have isolated epidermal and dermal DC from the skin of HLA-DR4+ patients and loaded them for 4h with NP, p24 peptide or p24NP before co-culture with HLA-DR4-restricted HIV-1 p24Gag (271-290)-specific CD4+ T cell clones (Moris et al., 2006). Although clonal activation was strictly antigen-dependent, the conjugation to NP did not significantly improve epidermal DC-mediated CD4+ T cell stimulation as evidenced by similar levels of IFN-γ production (Fig. 6b, lower panels). In contrast, p24NP-loaded dermal DC were significantly more efficient at inducing IFN-γ production from HIV-specific CD4+ T cell clones compared to dermal DC loaded with p24 peptide alone (Fig. 6b, upper panels). These data therefore strongly support the notion that NP-mediated antigen delivery into dermal DC subtypes substantially enhances antigen-specific CD4+ T cell priming, a critical step in the initiation of a coordinated immune response to vaccination.

Discussion

DC are critical mediators of cellular and humoral responses; they play a major role in eliciting immune responses to vaccines. Vaccination strategies that exploit the ability of DCs to capture, process and present antigens are attractive because they hold the potential to elicit coordinated innate and adaptive immune responses. The use of antigen-loaded MDDCs in this context has been bolstered by recent findings showing that these cells can be generated *in vivo* from tissue monocytes under inflammatory conditions (Dhodapkar et al., 1999).

Although most vaccines are currently administered by intramuscular injection, a growing interest in intradermal antigen delivery holds several potential advantages evidenced by recent scientific reports claiming an under-appreciated skin-mediated immunity due to the broad and diverse network of resident dermal and epidermal DC. Indeed, there are accumulating evidence suggesting that the skin may act as a major pathway leading to the establishment of enhanced immunity to exogenous protein antigens (Seneschal et al., 2012). ID vaccines are administered into the skin by injection, where the dermal and epidermal compartments contain a large and diverse network of DC. Skin-derived DC remain in an immature state whilst in the skin (Garrett et al., 2000; Pierre and Mellman, 1998). Once activated by various stimuli, they migrate to the draining lymph nodes where they subsequently activate CD4+ and CD8+ T cells. It has been suggested that LC are responsible for the development of cytotoxicity and dermal DC for the generation of the antibody response (Romani et al., 2012). For these reasons, we explored minimally invasive and controlled ID antigen delivery with the aim of informing the development of an effective vaccine against HIV-1.

Nanotechnology offers the opportunity to design nanoparticles varying in structure, size, shape and surface properties, to assist targeting of antigens to resident immature DC. To optimize antigen delivery to skin-resident DC, we used a nanoparticles comprising a hydrophobic rubbery core of cross-linked polypropylene sulfide surrounded by a hydrophilic corona of PEG functionalized for HIV-1 derived p24 Gag peptide (p24NP) delivery (Reddy et al., 2006; Reddy et al., 2007). Our results demonstrate a successful uptake of p24NP by MDDC. Although DC activation by the “adjuvant” effect

of the nanoparticle alone could be desirable, immature DC are capable of effective capture and process of the NP-associated antigen. p24NP DC uptake does not induce DC maturation as opposed to LPS or poly(I:C) adjuvants known to induce CD83 maturation marker expression and TNF-α production. This is a critical asset, as it allows the use of any compatible adjuvant without interference from the nanoparticle formulation.

Using HIV-1-specific CD4 and CD8+ T cell clones, we have demonstrated that p24NP are efficiently captured, and the derived HIV-1 p24 Gag-derived epitopes processed and presented by DC to T cells. We show that CD4 and CD8 IFN-γ responses can be detected at very low doses of p24NP, and with similar amplitude when comparing to MDDC and dermal-like DC. Both DC subtypes are able to stimulate potent specific helper CD4 and cytotoxic CD8 T cell responses as evidenced by the large amounts of IFN-γ -produced. Additionally, our data shows that p24NP can generate a polyclonal CD4+ T cell response independently of the type of DC used. Similarly, when CD4+ T cells were primed with p24NP-loaded DC, strong IFN-γ and IL-17 responses were observed with HIV-1-infected MDDC or MDLC. As vaccination aims to generate a robust antibody response, our investigation further shows increased IgG1 and IgG2 production upon immunization with p24NP, suggesting that our proposed p24NP formulation could be adapted to induce specific immunity against HIV-1 (Walker et al., 2011).

Intradermal (ID) vaccines deliver antigens to the skin where they encounter skin DC directly as well as lymph node-resident DC after their migration from the skin to the lymph nodes. It has been shown that antigen delivery to skin DC can be enhanced using nanoparticles, as particulate adjuvants and antigens are taken up more efficiently than soluble forms (Lambert and Laurent, 2008), leading to more efficient targeting of immunocompetent cells to the lymph node resident. ID vaccination has several potential advantages over other forms of vaccination. Delivery is more superficial, potentially avoiding the use of hypodermic needles, and can, in the case of some vaccines, induce more potent immune responses. ID delivery can be as effective as intramuscular (IM) vaccines at lower doses, which can decrease the cost of vaccine manufacturing and extend vaccine supply. Several technical obstacles have however slowed down the development of ID immunization. In parallel, the recent development of microneedle and

micro-injection devices has made intradermal immunization more accessible, potentially circumventing the need for medical personnel to administer the vaccine (Prausnitz et al., 2009). Dry coating the antigen formulation onto the surface of solid microneedles offers further advantages including increased antigen stability leading to improved shelf life and are less dependent on temperature-controlled supply chains, rendering these formulations much more appropriate for commercialization in developing countries (Wang, 2000).

Our data show that microneedles can readily deposit coated nanoparticles in the skin epidermis, and more effectively the dermis, as the coating covers the length of the microneedle shaft. Similarly, p24NP vaccination through ID delivery does not activate or stimulate dermal or epidermal DC, but provides sufficient signal to elicit T cell HIV-1-specific IFN-γ response against HIV-1.

In summary, our findings demonstrate the efficacy of a nanoparticle-based vaccine formulation delivered via intradermal route. We speculate that the DC ability to migrate to lymph nodes and intestinal mucosa may provide a way to stimulate "hard-to-reach" sites of latently infected cells. As our system of nanoparticle intradermal delivery allowed us to explore the unique properties of this formulation with dermal DC and LC, one of the important remaining questions is which DC subset should be targeted, and what would be the consequences at the immunological level and on the efficacy of the vaccine(Chu et al., 2012). In addition LC have been suggested to be more potent than dermal DC in CD8+ T cells priming (Artyomov et al., 2015; Banchereau et al., 2012; Zaric et al., 2015). Although our data could be limited by the endogenous capacity of HIV-1-specific T cell clones to produce cytokines, our results show comparable levels of IFN-γ and IL-17 production suggesting similar stimulatory potency of these DC subsets.

Complexing antigens with PPS-NP creates a particulate form of the antigen that offers many advantages such as antigen preservation from proteolytic enzymes, preferential distribution of antigens to APCs, higher efficiency of antigen loading, prolonged presentation of the processed antigen by DC, improved shelf life of the antigen for long-term storage, and easy scale up for pharmaceutical production. This approach may prove valuable in the global effort to develop more effective immunization strategies.

Materials and Methods

Cells and reagents

All patient samples and cell protocols were approved by Cardiff University Research Ethics Committee.

Primary cells were purified from peripheral blood mononuclear cells (PBMC) of healthy donors by magnetic-activated cell sorting according to the manufacturer's instructions (Miltenyi Biotec). CD14+ monocytes were purified and cultured for 5 days in complete IMDM medium supplemented with GM-CSF + IL-4 to generate immature monocyte-derived DC (MDDC), or with TGFβ + to generate monocyte-derived LC (MDLC). CD141+ dermal-like DC were differentiated from MDDC cultured in the presence of Vitamin D3 as previously described (Chu et al., 2012; Nestle et al., 1993). Mature DC were purified using Blood DC Isolation Kit II. CD4+ T cells were enriched by negative selection and maintained in the presence of IL-2. In some experiments, 1μg/ml LPS (Sigma) was used as positive control.

Skin DC isolation

Human skin samples were obtained from female patients undergoing mastectomy or breast reduction surgery under informed written patient consent and local ethical committee approval. Skin was prepared for use by removal of subcutaneous fat tissue by blunt dissection. Epidermis and dermis were collected by first cutting 300μm thick skin samples with a dermatome. Following incubation in Dispase II (Sigma), epidermis and dermis were separated. LC and dermal DC were collected by walkout of the epidermis and dermis respectively. LC and dermal DC were purified using magnetic beads.

p24NP synthesis

PPS-NP were synthesized, and conjugated with p24 peptide as previously described (Hirosue et al., 2010).

T cell clones

The following T cell clones were used: Ox97 clone 10, specific for HLA-DR1-restricted HIV-1 p24 Gag-derived epitope FD20 (residues 294-313) (Scriba et al., 2005); N12, specific for HLA-DR4-restricted HIV-1 p24 Gag-derived epitope NK20 (residues 271-290) (Moris et al., 2006); and clone 6, which recognizes the HLA-B57-restricted HIV-1 p24 Gag-derived epitope TW10 (residues 108-117) (Feeney et al., 2005).

Virus stock production

Viral stocks were generated by transient transfection of HEK293T cells using plasmids encoding full length X4-tropic HIV-1 (pR9 and pNL4.3). Infectious titers of viral stocks were quantified using a p24 Gag ELISA kit (Biological Products Core Laboratory).

Infection and transfer

DC were infected with 5ng p24Gag HIV equivalent by spinoculation, and washed immediately before initiating the coculture with CD4 T cells at 1:2 ratio.

Microneedle array

Microneedle arrays (5 microneedles of 750μm length) were fabricated from stainless steel sheets using wire electrical discharge machining (EDM). The arrays were subsequently electropolished under a current of 1.8 mA/mm^2 at 70˚C adapted from a method previously described (Gill and Prausnitz, 2007). Fluorescent red sulfate-modified polystyrene nanoparticles (mean diameter 0.1μm; Sigma-Aldrich) in an aqueous formulation were dip-coated onto microneedles at room temperature as previously described (Chong et al., 2013).

Coated microneedle arrays were inserted into human skin explants and held in place for 60s to allow dissolution of the formulation from the microneedle surface and deposition into the skin. The treated skin area was then excised, embedded in OCT embedding media and stored at -80ºC prior to sectioning. Sections of 10μm thickness were obtained using a Cryotome FSE (Thermo Scientific).

Flow cytometry

Productive infection was measured by flow cytometry analysis of HIV-1 p24 Gag+ cells: fixed and permeabilized cells were stained with mouse KC57-FITC (Beckman-Coulter). T cells and DC were stained with mouse HB15a anti-CD83 (Beckman Coulter), mouse HI149 anti-CD1a, mouse SK7 anti-CD3 (BD Biosciences), and mouse UCHL1 anti-CD45RO (eBioscience). Cytokines were measured by intracellular staining with mouse Mab11 anti-TNF-α, mouse 4S.B3 anti-IFN-γ, mouse 2A3 anti-IL-2 and mouse 033-782 anti-IL-17 (BD Biosciences).

Enzyme-linked immunosorbent assay

Purified B cells were added to DC-T cells cocultures for 12~14 days. Supernatants were harvested and diluted from 1/5 to 1/200. IgM (eBioscience) and IgG (Novex) were measured by ELISA according to manufacturer's recommendation.

Immunohistochemistry

After fixation in acetone at -20ºC, skin sections were stained with mouse anti-CD207-PE (Beckman Coulter) and Hoechst to visualize the epidermal LC and nuclei respectively. Images were captured using a Leica DM IRB epifluoresce miscroscope with Retiga Exi Fast digital camera (QImaging). Image post-processing was performed using ImageJ software.

The authors declare that they have conflict of interest. The nanomaterials used in this study are subject to patents filed by the Ecole Polytechnique Fédérale de Lausanne (EPFL), and JAH and MAS are inventors on those patents as well as own equity in a company that has licensed those patents.

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Figure Legend

Figure 1: p24NP rapid uptake by DC does not induce early maturation

a: MDDC were incubated with 0.1 or 1μg/ml p24NP for 24h and collected at the indicated time points up to 24h. The uptake of p24NP was measured by anti-p24 intracellular staining.

MDDC were incubated overnight with various concentrations of p24NP. CD83 and TNF-α were stained and analyzed by flow cytometry. Unconjugated nanoparticles and 1μg/ml LPS were used as negative and positive controls respectively. Statistical analysis was performed with MDDC (**b**) and dermal-like DC (**d**). Differences were not statistically significant. **c** is a representative flow cytometry analysis of TNF-α staining with 0.5μg/ml NP or p24NP, and 1μg/ml LPS used.

Figure 2: anti-p24 CD8 specific response to p24NP-loaded DC

p24 and p24NP-loaded autologous MDDC (**a, c**) or dermal-like DC (**b, c**) were cocultured overnight with TW10-specific CD8+ T cell clones. IFN-γ production was measured by intracellular staining on CD3+ cells and analyzed by flow cytometry. *: $p<0.05$, **: $p<0.01$, ***: $p<0.0001$.

Figure 3: anti-p24 CD4 specific response to p24NP-loaded DC

MDDC (**a, c**) or dermal-like DC (**b, d**) were incubated with various concentrations of p24 or p24NP, and cocultured with Ox97 CD4+ T cells clones overnight. pOx97 specific peptide is used a positive control.

IFN-γ production in CD3+ cells was measured by intra-cellular staining and analyzed by flow cytometry.

Figure 4: p24NP priming induces CD4 T cell response to HIV-1 infection

CD4+ T cells were stimulated with p24/ p24NP-loaded DC for 2 rounds. At the end of the second round, IFN-γ production was measured by flow cytometry after re-stimulation (**a, b**). **a**: statistical analysis. **b**: representative experiment. CD4+ T cells

immunized as in **a** with whole blood DC, then cocultured with MDLC or MDDC infected with HIV-1 pR9 for 6 days. IFN-γ (**c**) and IL-17 (**d**) production was tested after re-stimulation with PMA/ Ionomycin. *: $p<0.05$

Figure 5: p24NP induces a strong antibody response

a: CD4+ T cells were immunized as in Fig. 4. and co-cultured with autologous B cells. After 12~14 days, supernatants were tested for antibody production by ELISA assay. Intradermal microneedles were coated with yellow-fluorescent-coated nanobeads, and inserted into skin explants for 60 seconds. Frozen sections were stained with CD207 anti-langerin antibody (red), Hoechst 33342 for nucleus staining (blue). **b-c** show 2 representative images. Scale bar = 100μm.

Figure 6: p24NP delivered into skin-resident DC stimulates CD4+ T cells

a: Epidermal and dermal DC were purified from skin explants and treated with NP, p24NP or LPS for 24h, and then TNF-α secretion was measured by intracellular staining. N12 CD4 T cells were then cocultured with dermal or epidermal DC overnight in the presence of brefeldin A and stained for IFN-γ (**b**). (**c**) Corresponding data are shown for the irrelevant peptide and N12 positive peptide control.